Exhibit 10

Photodynamic therapy of acne vulgaris using 5-aminolevulinic acid versus methyl aminolevulinate

Stine Regin Wiegell, MD, and Hans Christian Wulf, MD, DSc
Copenhagen, Denmark

Background: Recent studies have shown that photodynamic therapy (PDT) is effective in the treatment of acne vulgaris. No studies have compared the treatment effect of aminolevulinic acid—PDT (ALA-PDT) and methyl aminolevulinate—PDT (MAL-PDT).

Objective: We sought to compare the treatment effect and tolerability of ALA-PDT versus MAL-PDT in the treatment of acne vulgaris in a controlled randomized investigator-blinded trial.

Methods: Fifteen patients with at least 12 facial inflammatory acne lesions had one split-face PDT treatment with MAL and ALA.

Results: Twelve weeks after treatment we found a 59% decrease in inflammatory lesions from baseline, with no significant differences in effectiveness between the two treatments. All patients experienced moderate to severe pain during illumination and developed erythema, pustular eruptions, and epithelial exfoliation after treatment, which were more severe and uniform in the ALA-PDT-treated area.

Limitations: The study is paired and controlled, but the results should be evaluated with consideration given for the number of participating patients.

Conclusion: PDT appeared to be an effective treatment for inflammatory acne vulgaris with no significant differences in the response rate between ALA-PDT and MAL-PDT. ALA-PDT resulted in more prolonged and severe adverse effects after treatment. (J Am Acad Dermatol 2006;54:647-51.)

Topical photodynamic therapy (PDT) is a new method for treatment of skin cancer and precancerous skin lesions. Recent studies have suggested that PDT is also effective in treatment of acne vulgaris.[1-3]

PDT is based on activation of light-sensitive molecules (photosensitizers), which form cytotoxic oxygen radicals, causing tissue injury and cell death.[4] 5-Aminolevulinic acid (ALA) is often used in topical PDT. ALA will convert in situ, via the heme cycle into protoporphyrin IX (PpIX), an extremely active photosensitizer activated by red light.[4]

Since ALA is a hydrophilic molecule, its penetration through cellular membranes and into the interstitial space of tissues is limited. Methyl aminolevulinate (MAL) is an ester of ALA with enhanced lipophilicity. MAL is de-esterified into ALA by intracellular enzymes. MAL should be expected to penetrate more easily and deeper into the targeted lesion.[5] Studies have shown that MAL is more selective toward abnormal skin lesions compared with ALA.[6] No studies, to our knowledge, have compared the treatment effect of ALA-PDT and MAL-PDT.

Patients undergoing PDT frequently experience moderate to severe pain in the treatment area during and after illumination. We have previously shown that MAL-PDT is less painful than ALA-PDT when treating normal skin.[7]

Abbreviations used:

ALA: 5-aminolevulinic acid
IQR: inter-quartile range
MAL: methyl aminolevulinate
PDT: photodynamic therapy
PpIX: protoporphyrin IX

From the Department of Dermatology, Bispebjerg Hospital, University of Copenhagen.
Funding sources: None.
Conflicts of interest: None identified.
Reprint requests: Stine Regin Wiegell, Department of Dermatology, D92, Bispebjerg Hospital. Bispebjerg Bakke 23, DK-2400 Copenhagen NV. E-mail: srw01@bbh.hosp.dk.
0190-9622/$32.00
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doi:10.1016/j.jaad.2005.12.033

The aim of this study is to compare the treatment effect and tolerability of ALA-PDT versus MAL-PDT when treating acne vulgaris.

METHODS

Patients

Fifteen patients participated in the study after written informed consent had been obtained. All patients were to be older than 18 years of age and should have a total of at least 12 inflammatory facial acne lesions. The patients were to have no history of topical or oral acne treatment within 4 months of study initiation and no oral retinoid treatment within 1 year. The protocol was approved by the Ethics Committee of Copenhagen and Frederiksberg (KF 01-177/04).

Treatment

Patients got one full-face PDT treatment (avoiding the eye area, nose, and lips) with ALA cream on one side of the face and MAL on the other side.

We used a commercial MAL cream (Metvix, PhotoCure ASA, Oslo, Norway). The ALA cream was produced by our hospital pharmacy as a 20% δ-aminolevulinic acid hydrochloride (Sigma Chemical Company, St Louis, Mo) in a Metvix-placebo cream. The application side of the two creams was randomized before the study. The patients and the primary investigator were blinded to the creams.

Approximately 2 g of each cream was applied and covered with light impermeable dressing for 3 hours, and both treatment areas were illuminated simultaneously with red light (Aktilite, PhotoCure ASA). The total dose was 37 J/cm^2 and the fluence rate of 34 mW/cm^2, which is half of the fluence rate normally used with the Aktilite. The fluence rate was reduced in an attempt to lessen the pain during illumination.

Evaluation

The evaluating dermatologist counted the number of different acne lesions before treatment and at 6 and 12 weeks after treatment. The lesion counts were taken separately from the left and right side of the face by using a face-counting template,[8] excluding the nose, lips, and the areas surrounding the eye. The dermatologist also assessed the global grade of acne severity.[9] The evaluating dermatologist was blinded to the creams.

Patients were asked to evaluate the pain during illumination and after treatment. Pain was assessed by means of a numeric scale ranging from 0 to 10, in which 0 is no pain and 10 is worst imaginable pain.

The amounts of PpIX in the treated areas were measured using a fluorescence camera (Medeikonos PDD/PDT, Medeikonos AB, Gothenburg, Sweden). Each image was calibrated using a fluorescence standard, and the mean amount of PpIX fluorescence in each image was calculated by means of a Matlab program.

We used nonparametric statistics, Wilcoxon signed rank test, and Spearman correlation to compare data.

RESULTS

Fifteen patients were treated with PDT. One patient did not appear at the 12-week follow-up and one patient did not appear for any follow-up. These two patients are not included in the primary outcome analysis.

Lesion counts of noninflammatory and inflammatory acne lesions are seen in Table I. Before treatment there was significantly more inflammatory lesions in the MAL-treated side of the face than in the ALA-treated side (P = .0049). This was a coincidence since the creams were randomized to either side of the face by lot before treatment.

The median decrease in numbers of inflammatory lesions from baseline to the 12-week follow-up were 13 (inter-quartile range [IQR] 7,16) on the MAL-treated side and 10 (IQR 4,15) on the ALA-treated side (Fig 1), giving a median percentage decrease of inflammatory lesions of 59% in both the MAL- and ALA-treated sides of the face.

There were no significant differences in absolute or percentage reduction of inflammatory lesions between the two treatments (P = .0803 and P = .455, respectively). There were no significant differences in noninflammatory lesions between the two treatments (P = .6355).

The median acne global severity grade was reduced from 2 to 1 in both the MAL-PDT– and ALA-PDT–treated sides of the face from before treatment to 12-week follow-up. There were no significant differences in global severity grade between the two treatments (P = .250).

At the 12-week follow-up the dermatologist found that 83% of the patients had a slight to marked improvement of their acne in the MAL-PDT side of the face and 75% in the ALA-PDT side of the face, but the difference was not significant (P = .375). The remaining patients had no change or worsening of their acne.

PpIX images showed significantly higher and more uniform PpIX fluorescence in the ALA-PDT side of face (Fig 2, *D* and *E*), with a median PpIX fluorescence in treatment area of 17591 arbitrary units (au) (IQR: 14,139 au, 20201 au) compared with 15,245 au (IQR: 14,008; 16,887 au) on the MAL-PDT side (P = .0078).

All patients experienced moderate to severe pain during illumination. There were no significant

Table I. Median lesion counts at baseline, 6-week, and 12-week follow-up on MAL-PDT–treated and ALA-PDT–treated sides of the face

Lesion counts	MAL-PDT Median (IQR)	ALA-PDT Median (IQR)	*P* value
Noninflammatory lesions			
Baseline	14 (6, 22)	17 (7, 21)	.24
6-week follow-up	21 (17, 31)	18 (13, 29)	.18
12-week follow-up	17 (9, 29)	20 (17, 38)	.052
Inflammatory lesions			
Baseline	19 (13, 27)	16 (11, 22)	.0049
6-week follow-up	8 (6, 14)	5 (3, 11)	.061
12-week follow-up	8 (3, 11)	5 (3, 11)	.080
Decrease from baseline at 12-week follow-up	13 (7, 16)	10 (4, 15)	.38

ALA-PDT, 5-Aminolevulinic acid–photodynamic therapy; *IQR*, interquartile range; *MAL-PDT*, methyl aminolevulinate–photodynamic therapy.

differences in maximal pain scores between the two sides during illumination ($P = .275$) with a median pain score of 7 (IQR: 4,9) on the ALA-PDT side of the face and 8 (IQR: 4,8) on the MAL-PDT side. Twenty-four hours after illumination the ALA-PDT side of the face was significantly more painful than the MAL-PDT side ($P = .0156$), with a median pain score of 1 (IQR: 0,4) on the MAL-PDT side and 2 (IQR: 1,7) on the ALA-PDT side.

We found a correlation between PpIX and pain during illumination on the ALA-PDT side of the face, but not on the MAL-PDT side ($P = .0073$ and $P = .172$, respectively).

After illumination edema and severe inflammation were seen in the treatment area (Fig 2, *B*). In the following days, a pustular eruption and epithelial exfoliation occurred. Cytologic examination of the content of the pustules showed large numbers of neutrophils but no sebum and no fungi or bacteria. Six patients developed yellow crusting and were treated with topical antibiotics to prevent infection. Twelve patients (80%) had more pronounced adverse effects after treatment on the ALA-PDT side of the face compared with the MAL-PDT side (Fig 2, *B*). The remaining 3 patients had no differences in adverse effects between the two sides of the face. Approximately half of the patients did not go to school or work the following days because of their appearance after treatment.

PDT was an efficient treatment of inflammatory acne vulgaris with no significant differences in response rate between ALA-PDT and MAL-PDT. The two treatments were equally painful during illumination but ALA-PDT resulted in more prolonged and severe adverse effects after treatment.

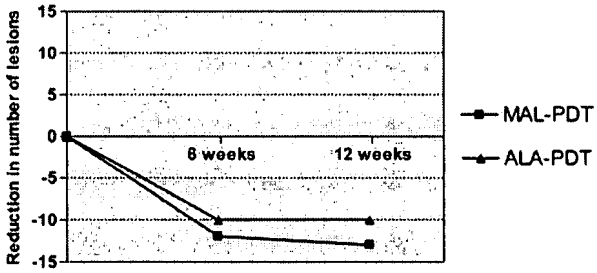

Fig 1. Median reduction in numbers of inflammatory lesion counts from baseline.

Because of the severe adverse effect using ALA, MAL-PDT is preferred when treating inflammatory acne vulgaris. However, the treatment regime should be optimized to diminish the adverse effects.

DISCUSSION

Topical and systemic antibiotics and isotretinoin are often very good treatment options for patients with acne vulgaris. However, many patients experience a need for a different treatment because of the transient effect and severe adverse effects. Previous studies have shown that PDT is very effective for acne vulgaris using either MAL or ALA.[1-3]

We used a split-face design, so the patients could serve as their own control. To ease the counting procedure, we used a template dividing the face into smaller areas. Eyes, nose, and mouth naturally separated the face in two halves, which made it easy for the patients to score the pain on the two sides.

In this study we have shown that ALA-PDT and MAL-PDT are equally effective in the treatment of inflammatory acne vulgaris. We found a 59% median reduction in inflammatory acne lesions 12 weeks after treatment in both the ALA-PDT– and the MAL-PDT–treated sides of the face.

These results are very similar to the 68% median reduction in inflammatory lesions found in an earlier study of MAL-PDT for acne vulgaris.[3] Although our patients were only treated once compared with two treatments in the earlier study, we did not perform curettage on the face before application of the creams, and the fluences during illumination were halved compared with the earlier study.

We found an increase in noninflammatory lesions after treatment, as seen in the earlier study. For 12 of the patients, lesion counts started 3 months before PDT. These lesion counts showed that the increase in noninflammatory lesions had started before the PDT treatment was performed, which indicates that the increase was not associated with the treatment.

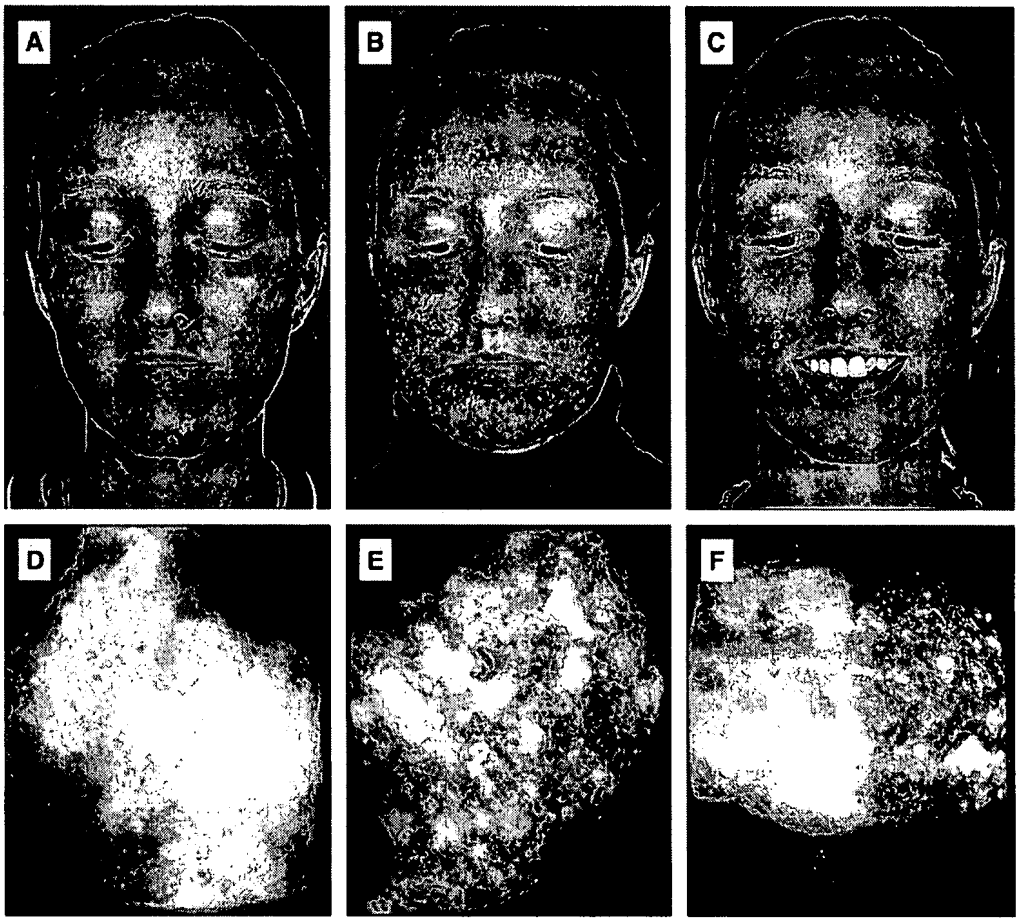

Fig 2. Clinical and fluorescence photographs from a patient treated with ALA-PDT on right side of face and MAL-PDT on left side. **A**, Before treatment. **B**, One day after first treatment, showing severe inflammation, edema, and pustules, especially on ALA-PDT–treated side of face *(right)*. **C**, At 12-week follow-up. **D**, Fluorescence photograph of right cheek treated with ALA for 3 hours. **E**, Fluorescence photograph of left cheek treated with MAL for 3 hours. **F**, Fluorescence photograph of forehead treated with ALA on right side and MAL on left side.

A study by Fritch et al[6] has shown that application of ALA will induce a higher accumulation of PpIX in both normal and lesional skin, but MAL is more selective toward diseased skin. We also found a higher accumulation of PpIX in the skin of the ALA-PDT side than in the MAL-PDT side of the face. Despite a lower overall fluorescence, the enhanced selectivity of MAL-PDT might explain the missing differences in clinical efficacy between the two creams.

We found no differences in pain scores during illumination between the two sides of the face. PDT of normal skin has shown that treatment with ALA is more painful than with MAL.[7] Treatment of diseased skin may lead to a high accumulation of PpIX using either MAL or ALA, whereas in normal skin the accumulation of PpIX is much higher after ALA application than after MAL. This may explain why we only found a difference in pain score between the two creams when treating normal skin.

There was a correlation between PpIX and pain in the ALA-PDT side of the face, but not in the MAL-PDT side. ALA induces a very homogeneous accumulation of PpIX and the more PpIX, the more severe pain during illumination. PpIX induced by MAL is distributed in spots, but the accumulation of PpIX can be very high in these spots. Activation of high amounts of PpIX in one spot may be as painful as activating a more homogeneous treatment area. This may explain why we did not find a correlation between PpIX and pain in the MAL-PDT side of the face.

ALA-PDT was associated with more severe adverse effects than MAL-PDT. This can also be explained by the higher accumulation of PpIX in normal skin, leading to a photodynamic effect in not only the inflammatory acne lesions but also in the

surrounding normal skin, thereby giving a more homogeneous and severe redness and swelling.

One patient developed severe swellings and erythema after treatment, especially on the ALA-PDT side of the face. Sterile pustular eruptions were also severe and were covering the entire forehead (both ALA-PDT– and MAL-PDT–treated areas). The redness faded after approximately 1 week on the MAL-PDT side of the face, whereas it persisted for more than 3 weeks on the ALA-PDT side of the face. A small superficial scar was seen over the cheekbone on the ALA-PDT side and baseline photographs verified that the scar had not been there before treatment. Despite the severe adverse effects, the patient was very content with the treatment and would consider further PDT in the future.

We thank technician Pia Eriksen for her practical help during the study, photographer Nis Kentorp for taking clinical photographs, and engineer Peter Philipsen for statistical advice during evaluation of the data.

REFERENCES

1. Hongcharu W, Taylor CR, Chang Y, Aghassi D, Suthamjariya K, Anderson RR. Topical ALA-photodynamic therapy for the treatment of acne vulgaris. J Invest Dermatol 2000;115: 183-92.
2. Pollock B, Turner D, Stringer MR, Bojar RA, Goulden V, Stables GI, et al. Topical aminolevulinic acid-photodynamic therapy for the treatment of acne vulgaris: a study of clinical efficacy and mechanism of action. Br J Dermatol 2004;151:616-22.
3. Wiegell SR, Wulf HC. Photodynamic therapy of acne vulgaris using methyl aminolevulinate—a randomized blinded controlled study. Br J Dermatol doi:10.1111/j.1365-2133.2005. 07107.x. Published online January 24, 2006.
4. Kennedy JC, Pottier RH. Endogenous protoporphyrin IX, a clinically useful photosensitizer for photodynamic therapy. J Photochem Photobiol B 1992;14:275-92.
5. Peng Q, Soler AM, Warloe T, Nesland JM, Giercksky K-E. Selective distribution of porphyrins in skin thick BCC after topical application of ALA-ME. J Photochem Photobiol B 2001; 62:140-5.
6. Fritsch C, Homey B, Stahl W, Lehmann P, Ruzicka T, Sies H. Preferential relative porphyrin enrichment in SK upon topical application of ALA-ME. Photochem Photobiol 1998;68:218-21.
7. Wiegell SR, Stender I-M, Na R, Wulf HC. Pain associated with photodynamic therapy using 5-aminolevulinic acid or 5-aminolevulinic acid methylester on tape-stripped normal skin. Arch Dermatol 2003;139:1173-7.
8. Lucky AW, Barber BL, Girman CJ. A multirater validation study to assess the reliability of acne lesion counting. J Am Acad Dermatol 1996;35:559-65.
9. O'Brian SC, Lewis JB, Cunliffe WJ. The Leeds revised acne grading system. J Dermatolog Treat 1998;9:215-20.